Integration of new approach methodologies for cosmetic safety decision making

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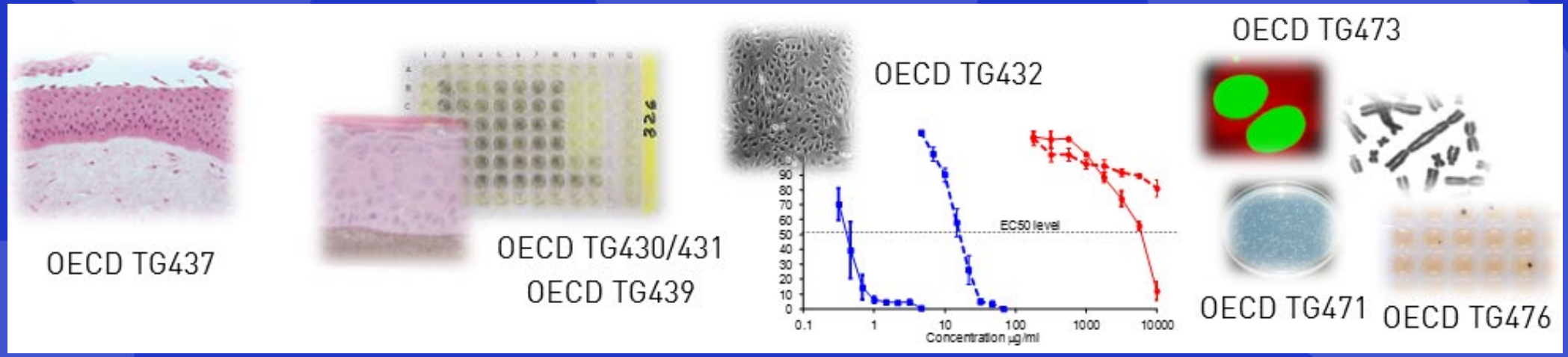
Use of Existing OECD *In Vitro* Approaches



Skin and eye irritation; skin sensitization; phototoxicity; mutagenicity… what about systemic toxicity?

Principles of NGRA from ICCR

4 Main overriding principles:

- The overall goal is a human safety risk assessment
- The assessment is exposure led
- The assessment is hypothesis driven
- The assessment is designed to prevent harm

3 Principles describe how a NGRA should be conducted:

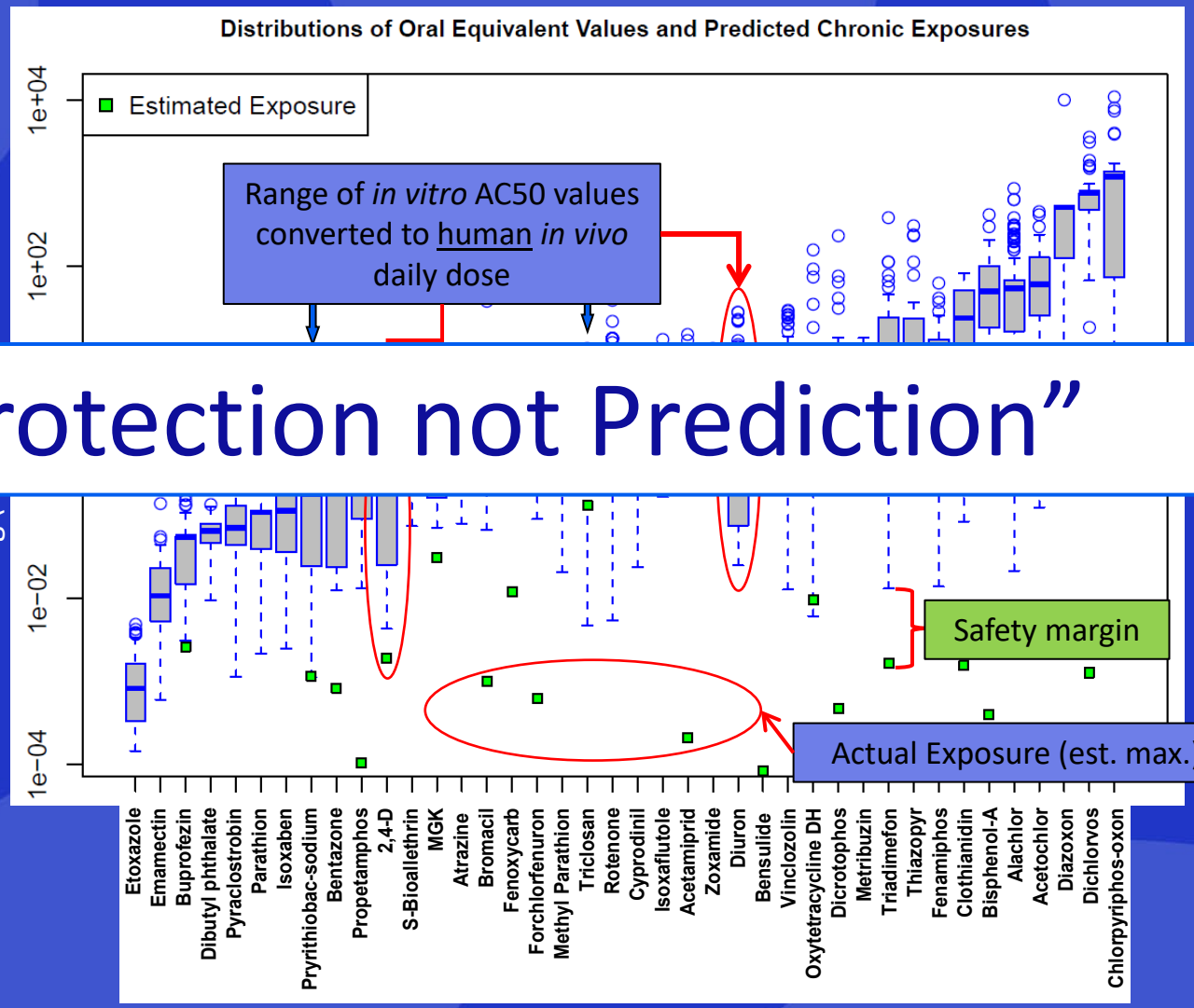
- Following an appropriate appraisal of existing information
- Using a tiered and iterative approach
- Using robust and relevant methods and strategies

2 Principles for documenting NGRA:

- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparently and documented

Dent *et al .,* (2018) *Comp Tox* 7:20-26

In Vitro Bioactivity vs Bioavailabilty



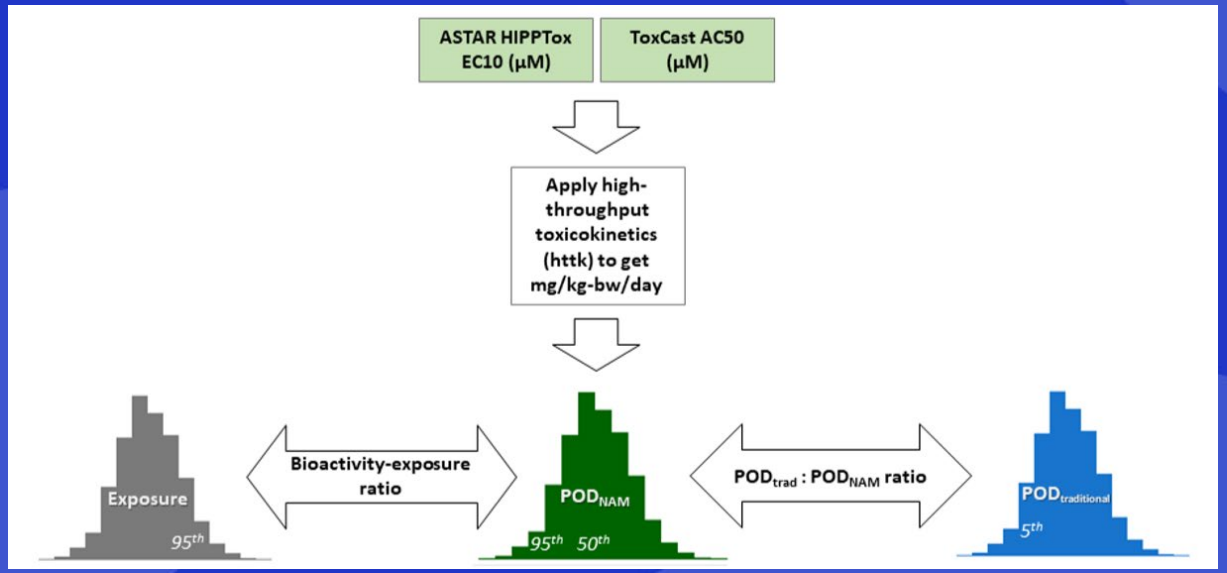
"Protection not Prediction"

Hepatic clearance and plasma protein binding determinations

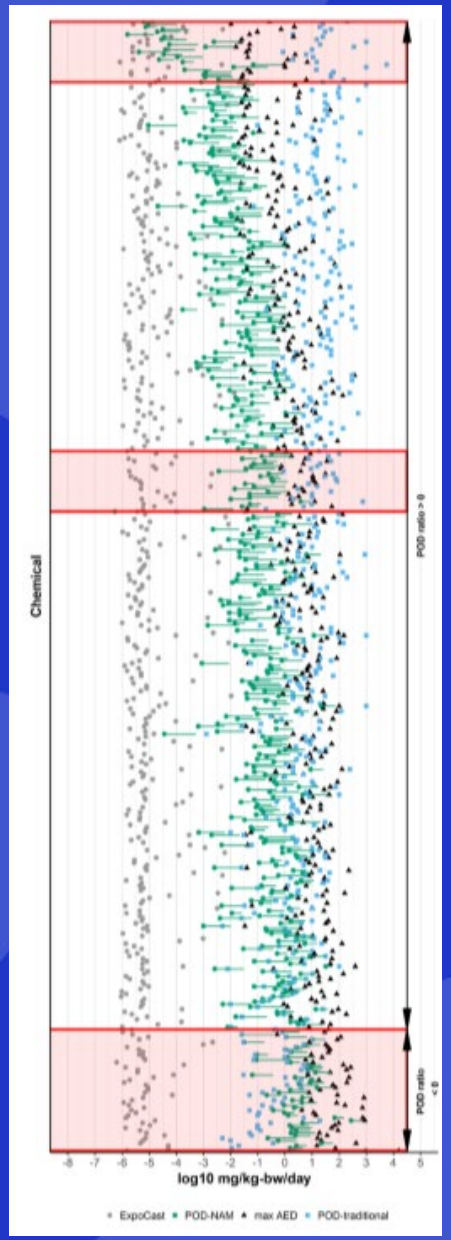
Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010

EPA, NTP, HC, A*STAR, ECHA, EFSA, JRC, RIVM…

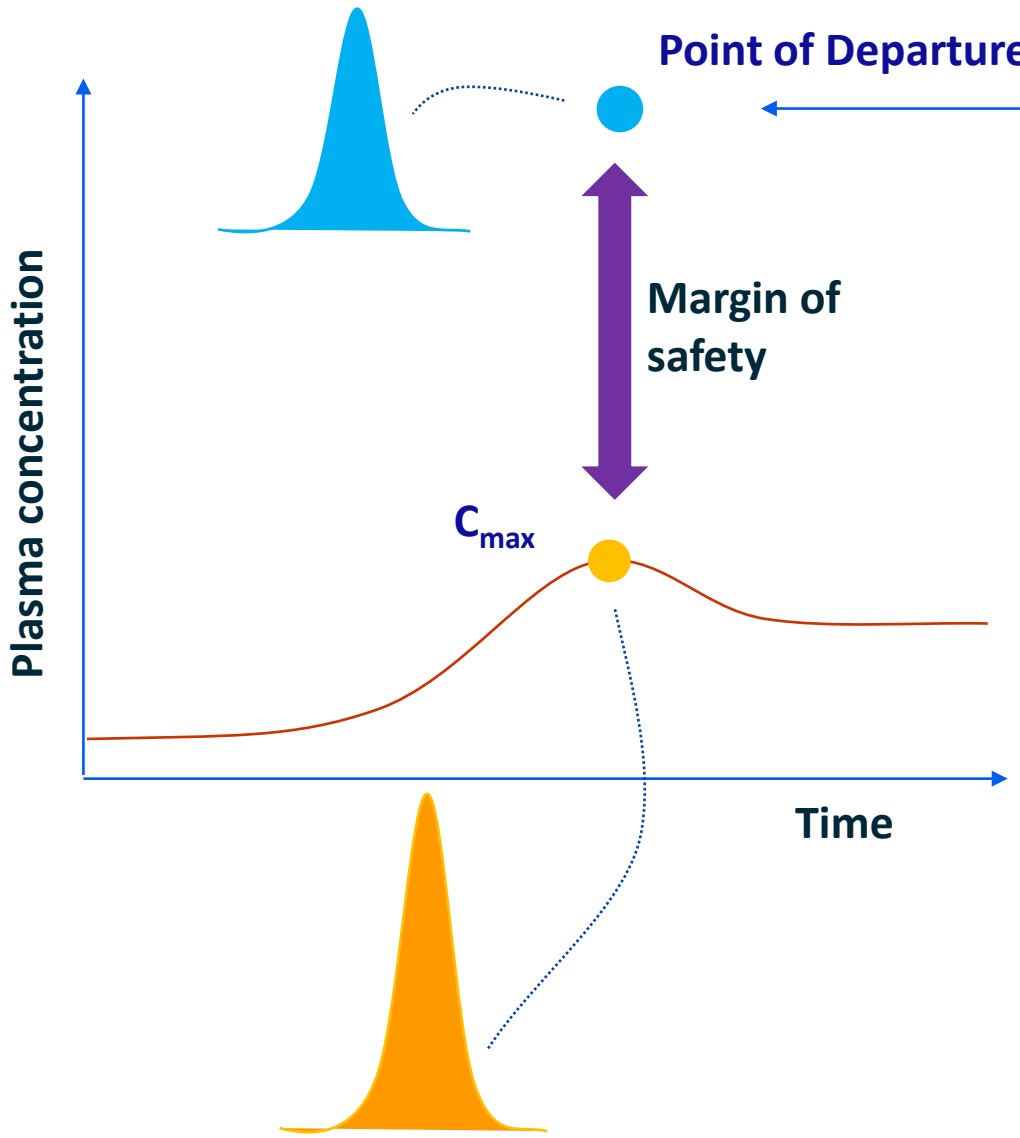


Katie Paul-Friedman *et al.* 2019 Tox Sciences, *October Issue*

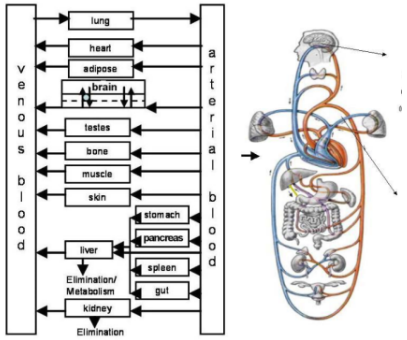


414/448 chemicals = *92% of the time this naïve approach appears conservative*

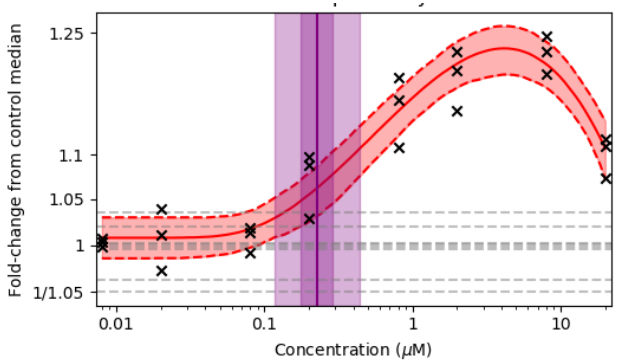
The Margin of Safety Approach



Exposure models (PBK, free/total concentration)



Point of departure derived from *in vitro* concentration-response



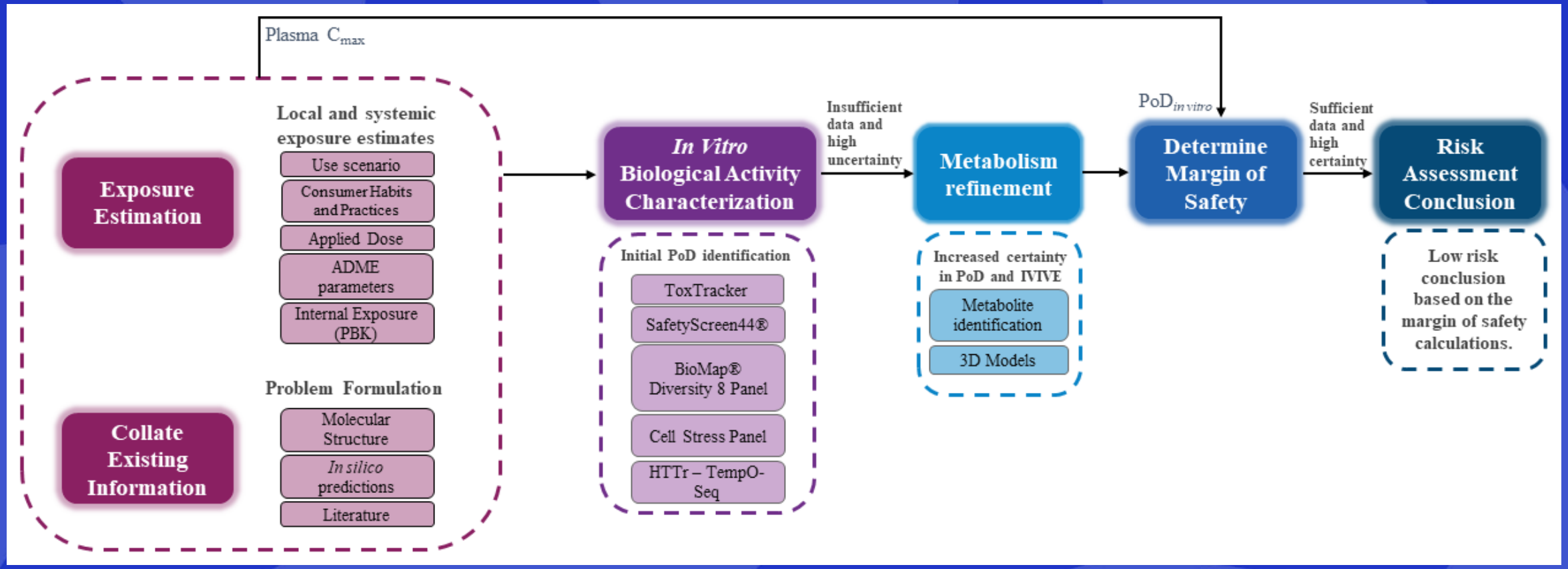
Case Study Approach… Imagine we have no data for: Coumarin

Baltazar et al., (2020) *Toxicological Sciences,* accepted

Safety assessment required for 0.1% coumarin in Body Lotion

Safety assessment required for 0.1% coumarin in Face Cream

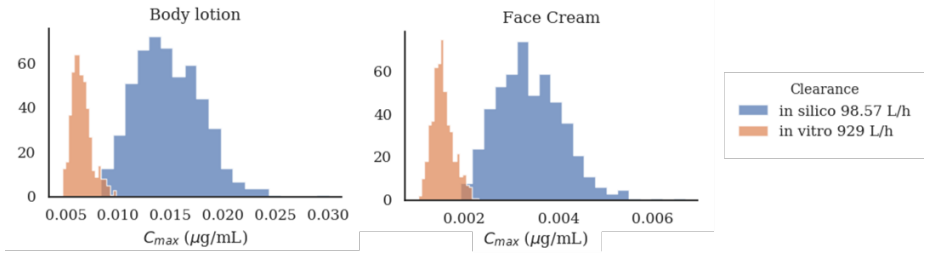
Case Study Framework



Systemic Bioavailability using PBK Modelling

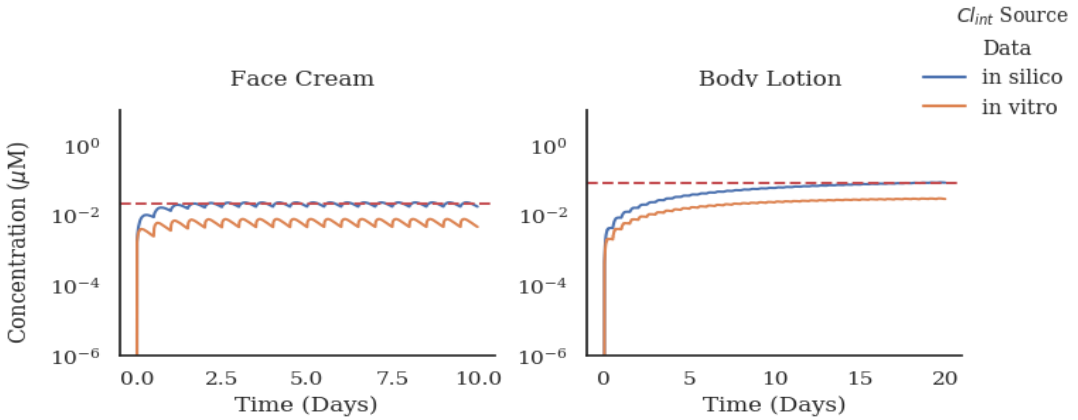
Key output parameters from uncertainty analysis:

Total Plasma C_{max} (µM)	Mean	Median	90th percentile	95th percentile	97.5th percentile	99th percentile
Face Cream	**0.0022**	**0.0021**	**0.004**	**0.0043**	**0.0046**	**0.005**
Body lotion	**0.01**	**0.01**	**0.018**	**0.019**	**0.02**	**0.022**



Uncertainty & Population Variability

0.1% Face cream & body lotion in Europe



Physiologically-based kinetic modelling using GastroPlus® v9.5.

Estimations based on experimental data (Clint, fup, bpr, solubility, LogP). Skin penetration parameters were fitted against skin penetration data.

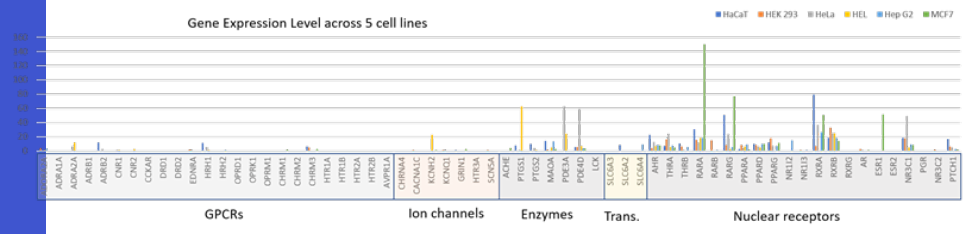
Moxon *et al* (2020) Toxicology in Vitro, **63** 104746

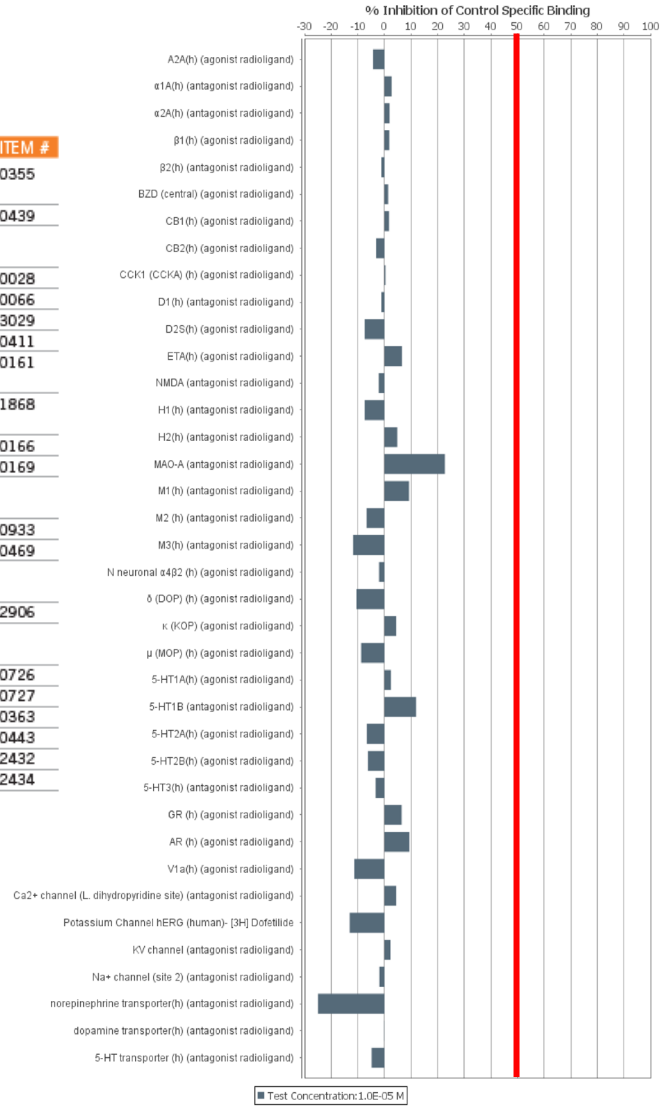
In Vitro Bioactivity: Safety Screen

eurofins | Cerep

Bowes et al 2012. Nature Reviews: Drug Discovery 11 909-922

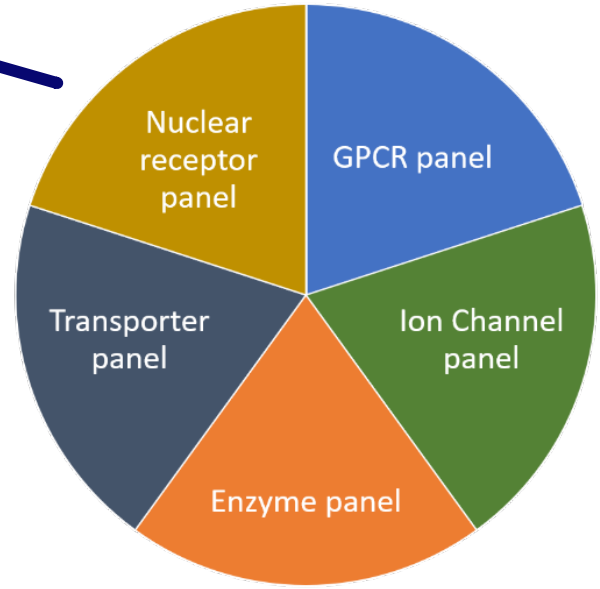
FAMILY	ASSAY	FORMAT	ITEM #
■ GPCR			
ADENOSINE	A_{2A}	•	0004
ADRENERGIC	$alpha_{1A}$		2338
	$alpha_{2A}$		0013
	$beta_1$	•	0018
	$beta_2$	•	0020
CANNABINOID	CB_1	•	0036
	CB_2	•	0037
CHOLECYSTOKININ	CCK_1 (CCK_A)	•	0039
DOPAMINE	D_1		0044
	D_{2L}	•	1322
ENDOTHELIN	ET_A	•	0054
HISTAMINE	H_1		0870
	H_2		1208
MUSCARINIC	M_1		0091
	M_2		0093
	M_3		0095
OPIOID & OPIOID-LIKE	$delta_2$ (DOP)	•	0114
	kappa (KOP)	•	1971
	mu (MOP)	•	0118
SEROTONIN	$5\text{-}HT_{1A}$	•	0131
	$5\text{-}HT_{1B}$		0132
	$5\text{-}HT_{2A}$	•	0471
	$5\text{-}HT_{2B}$	•	1333
VASOPRESSIN	V_{1a}	•	0159
■ TRANSPORTERS			
DOPAMINE	dopamine transporter		0052
NOREPINEPHRINE	norepinephrine transporter		0355
SEROTONIN	5-HT transporter		0439
■ ION CHANNELS			
GABA CHANNELS	BZD (central)	•	0028
GLUTAMATE CHANNELS	NMDA		0066
NICOTINIC CHANNELS	N neuronal α4β2	•	3029
SEROTONIN CHANNELS	$5\text{-}HT_3$		0411
Ca^{2+} CHANNELS	Ca^{2+} channel (L, dihydropyridine site)		0161
K^+ CHANNELS	hERG (membrane preparation)		1868
	K_v channel		0166
Na^+ CHANNELS	Na^+ channel (site 2)		0169
■ NUCLEAR RECEPTORS			
STEROID NUCLEAR RECEPTORS	AR	•	0933
	GR	•	0469
■ KINASES			
CTK	Lck kinase		2906
■ OTHER NON-KINASE ENZYMES			
AA METABOLISM	COX_1		0726
	COX_2		0727
MONOAMINE & NEUROTRANSMITTER	acetylcholinesterase		0363
	MAO-A		0443
PHOSPHODIESTERASES	PDE3A		2432
	PDE4D2		2434





All binding and enzymatic assay results were negative at 10 uM, including COX-1 and COX-2

No receptor/target-led pharmacological effect



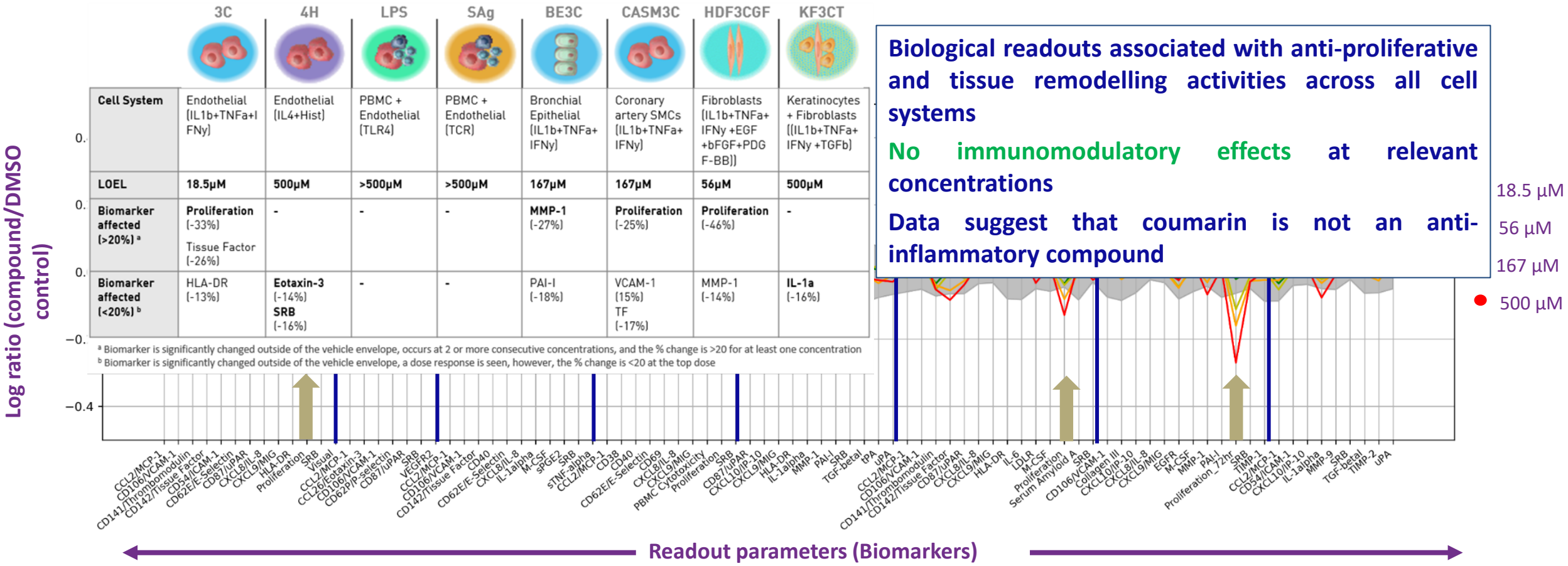
SafetyScreen44™ Panel

Immunomodulatory Bioactivity: BioMap® Diversity 8 Panel

BioMAP systems contain human primary cell types (or combinations) that are stimulated to replicate complex cell and pathway interactions of vascular inflammation, immune activation and tissue remodelling

	3C	4H	LPS	SAg	BE3C	CASM3C	HDF3CGF	KF3CT
Cell System	Endothelial (IL1b+TNFa+IFNy)	Endothelial (IL4+Hist)	PBMC + Endothelial (TLR4)	PBMC + Endothelial (TCR)	Bronchial Epithelial (IL1b+TNFa+ IFNy)	Coronary artery SMCs (IL1b+TNFa+ IFNy)	Fibroblasts (IL1b+TNFa+ IFNy +EGF +bFGF+PDGF-BB)]	Keratinocytes + Fibroblasts ([(IL1b+TNFa+ IFNy +TGFb)
LOEL	**18.5µM**	**500µM**	**>500µM**	**>500µM**	**167µM**	**167µM**	**56µM**	**500µM**
Biomarker affected (>20%) [a]	**Proliferation** (-33%) Tissue Factor (-26%)	-	-	-	**MMP-1** (-27%)	**Proliferation** (-25%)	**Proliferation** (-46%)	-
Biomarker affected (<20%) [b]	HLA-DR (-13%)	**Eotaxin-3** (-14%) **SRB** (-16%)	-	-	PAI-I (-18%)	VCAM-1 (15%) TF (-17%)	MMP-1 (-14%)	**IL-1a** (-16%)

[a] Biomarker is significantly changed outside of the vehicle envelope, occurs at 2 or more consecutive concentrations, and the % change is >20 for at least one concentration
[b] Biomarker is significantly changed outside of the vehicle envelope, a dose response is seen, however, the % change is <20 at the top dose



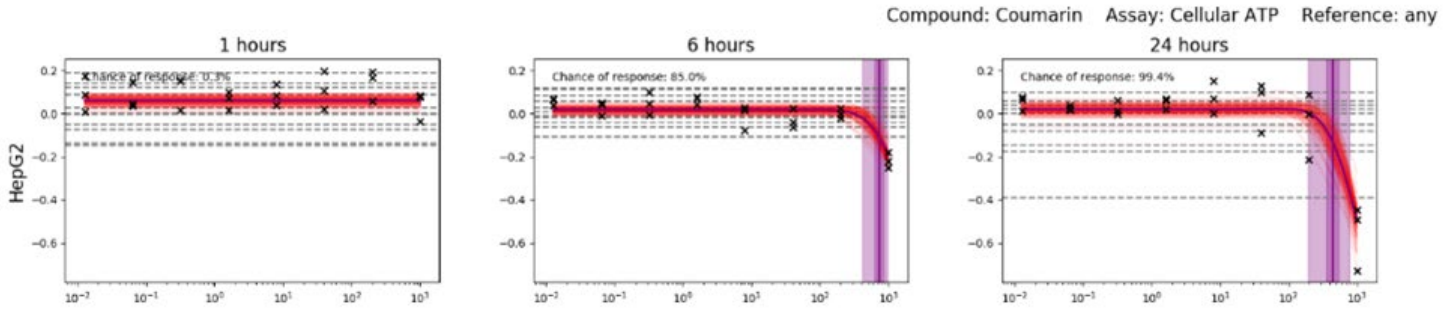
Biological readouts associated with anti-proliferative and tissue remodelling activities across all cell systems

No immunomodulatory effects at relevant concentrations

Data suggest that coumarin is not an anti-inflammatory compound

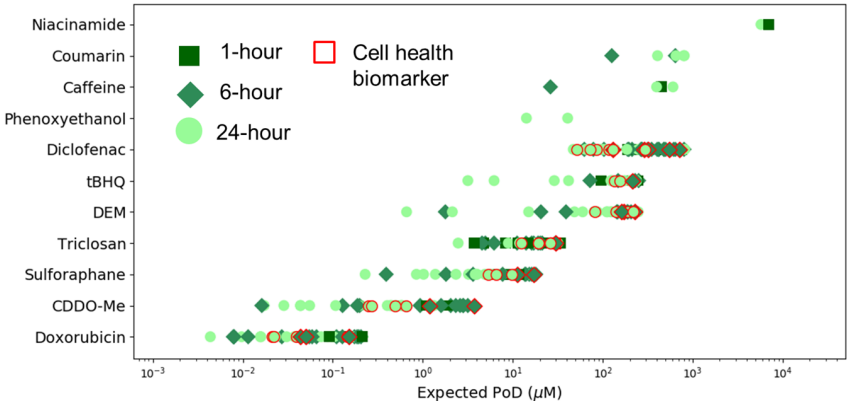
In Vitro Bioactivity: Cell Stress Panel

Hatherell et al., (2020) Toxicological Sciences, accepted



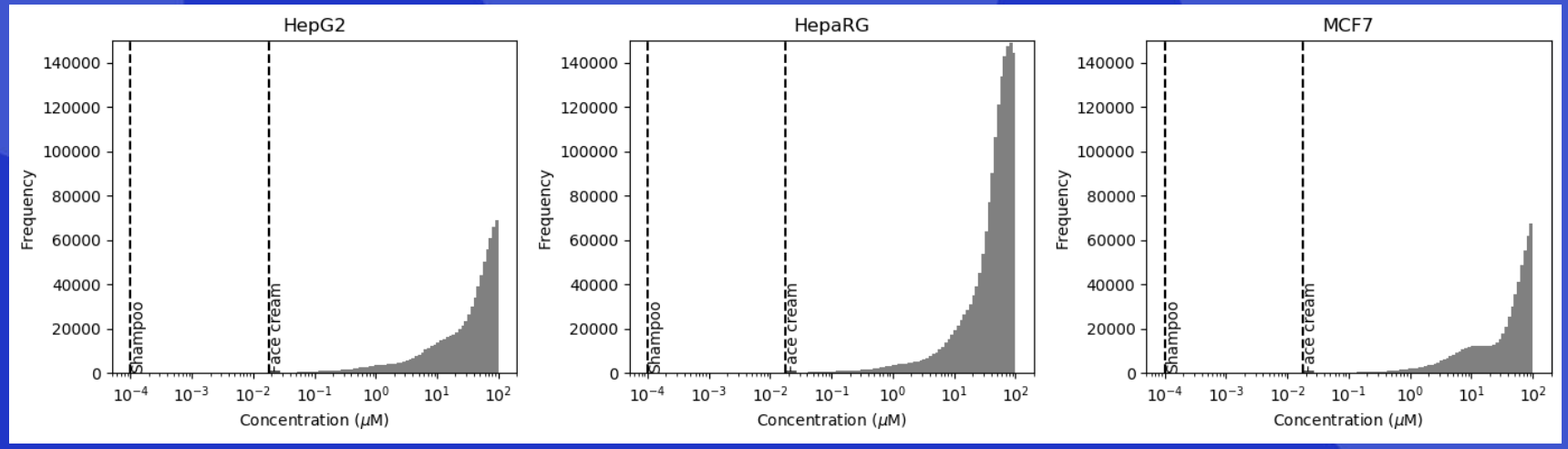
Biomarker	Stress pathway	PoD (2.5th percentile), µM	PoD (50th percentile), µM	PoD (97.5th percentile), µM	Effect
Cell count (72h)	Cell health	54	150	316	down
ATP (6h) **ATP (24h)**	Cell health	411 194	738 449	976 763	down
GSH (24h)	Oxidative stress	641	781	979	up
IL-8 (6h) **IL-8 (24H)**	Inflammation	8.8 343	52 698	123 974	down
Phosholipidosis (24h) **Phosholipidosis (72h)**	Cell health	289 285	605 588	949 915	down
LDH (1h)	Cell health	52	370	974	up
ICAM-1 (24h)	Inflammation	354	696	973	down
Steatosis	Cell health	59	659	974	up

Summary with PoD for cell stress biomarkers:



- **Coumarin not very active in comparison to known 'high risk compounds' like doxorubicin, diclofenac etc.**
- **Cell count, cellular ATP, GSH, IL-8, Phospholipids, LDH, ICAM-1 and steatosis showed a dose response**

In Vitro Bioactivity: Tempo-Seq Technology



- Coumarin dose range 0.001uM to 100uM
- 24 hour time point
- QC and normalisation in DESeq2
- BMDExpress2 applied to determine NOTEL (3 pathway approaches)

Cell Model	HepG2	MCF7	HepaRG 2D
Pathway Level Tests	(308 pathways)	(0 pathways)	(17 pathways)
20 pathways with the lowest pvalue Reactome	70	NA	58*
20 pathways with the lowest BMD Reactome	44	NA	58*
BMD of Reactome pathway with lowest BMD that meets significance threshold criteria	31	NA	38
Gene Level Tests	(1570 genes)	(47 genes)	(87 genes)
Mean BMD of 20 genes with largest fold change	6	3	54
Mean BMD of Genes between 25th and 75th percentile	17	1	59

Case Study Framework

Plasma C_{max}

Exposure Estimation

Local and systemic exposure estimates
- Use scenario
- Consumer Habits and Practices
- Applied Dose
- ADME parameters
- Internal Exposure (PBK)

Collate Existing Information

Problem Formulation
- Molecular Structure
- *In silico* predictions
- Literature

***In Vitro* Biological Activity Characterization**

Initial PoD identification
- ToxTracker
- SafetyScreen44®
- BioMap® Diversity 8 Panel
- Cell Stress Panel
- HTTr – TempO-Seq

Insufficient data and high uncertainty

Metabolism refinement

Increased certainty in PoD and IVIVE
- Metabolite identification
- 3D Models

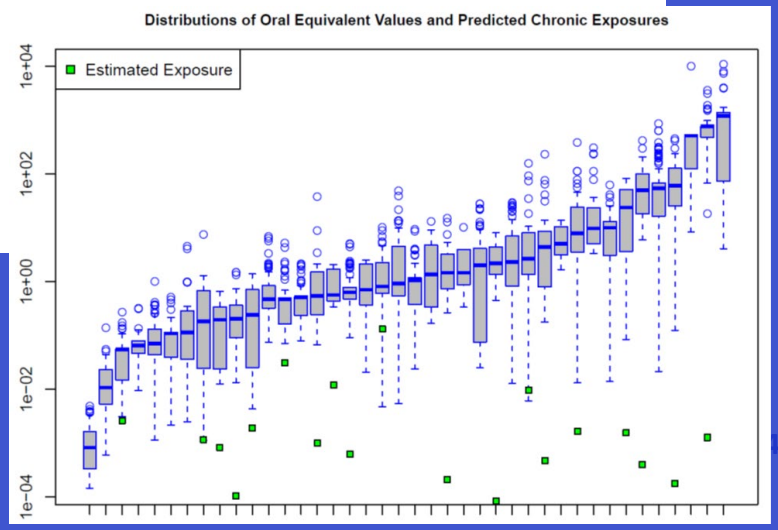
$PoD_{in\ vitro}$

Determine Margin of Safety

Sufficient data and high certainty

Risk Assessment Conclusion

Low risk conclusion based on the margin of safety calculations.



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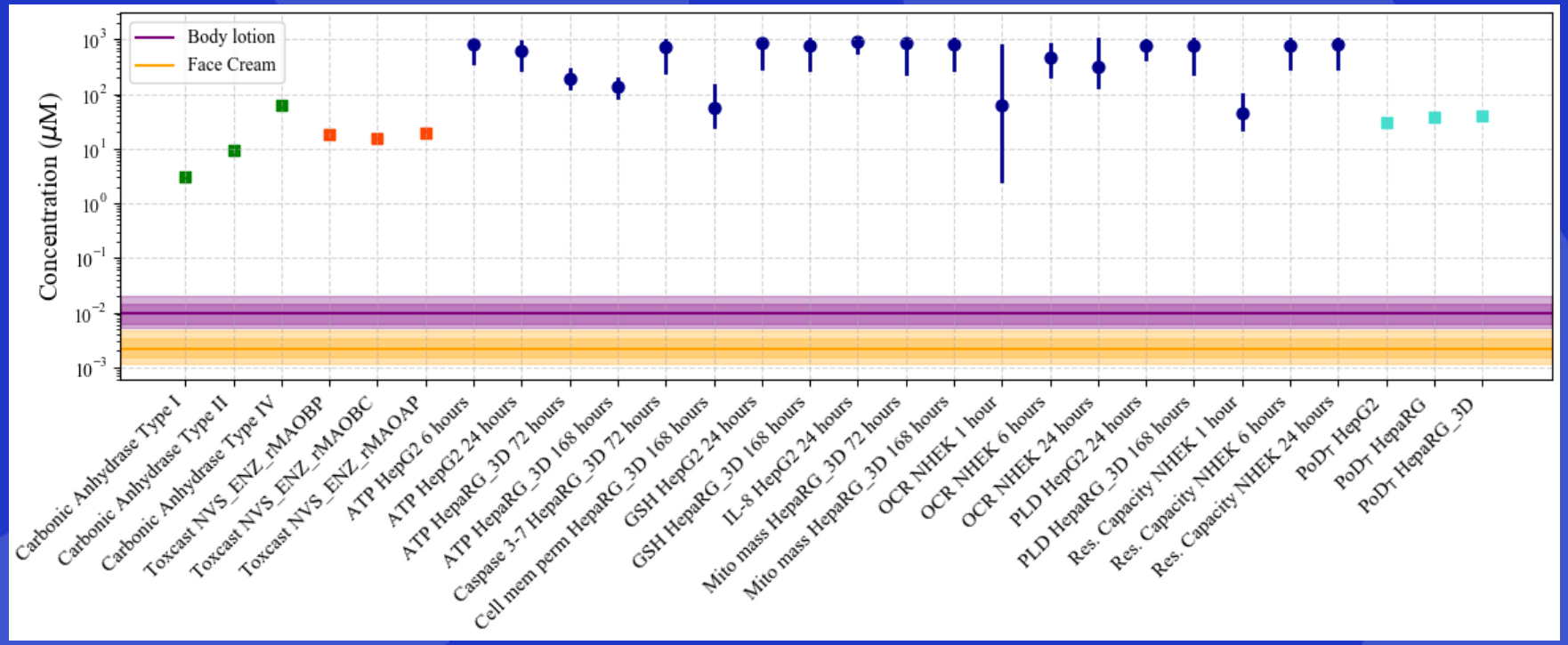
Margin of Safety considering PODs and Exposure

PoDs and plasma C_{max} (µM) are expressed as total concentration

PubChem **ToxCast** **Cell Stress Panel** **HTTr**



C_{max} expressed as a distribution:

- Line = median (50th percentile)
- Inner band = 25th-75th percentile
- Outer band = 2.5th-97.5th percentile (95th credible interval)

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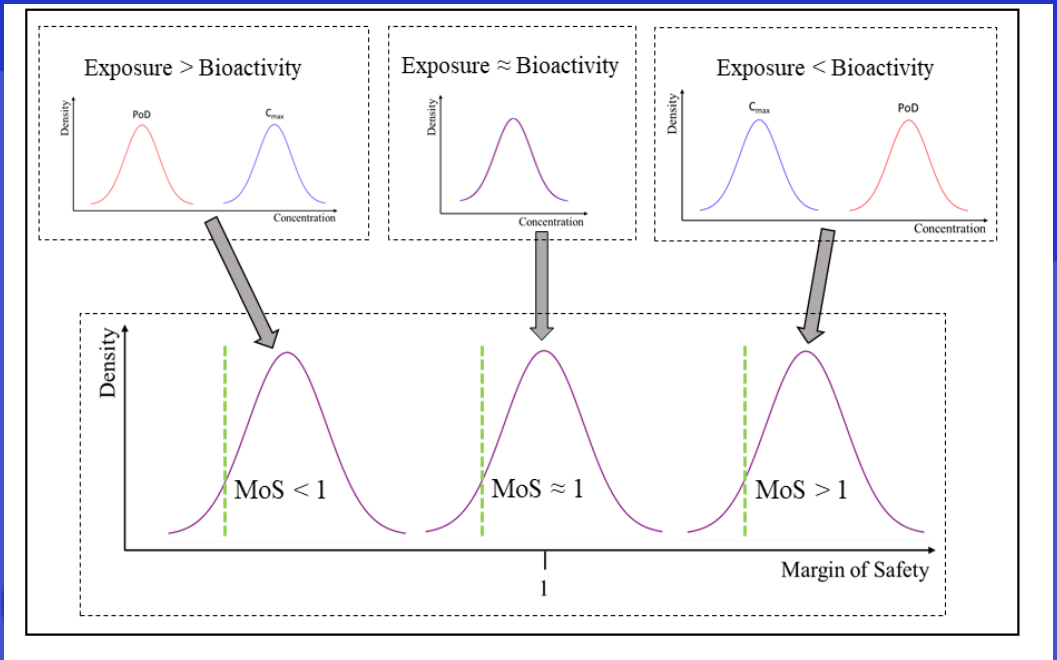
Application of *Ab Initio* Approach: Risk Assessment (NGRA)

Margin of safety is the **fold difference** between the **Cmax and the *in vitro* POD**



Technology	Cell line/ Enzyme/Biomarker	Face cream Min. 5th percentile MoS	Body Lotion Min. 5th percentile MoS
Cell stress panel	HepG2 (ATP, 24h)	96738	22048
Cell stress panel	NHEK (OCR 1h)	1330	**295**
HTTr	HepG2 (24h)	7223	1618
HTTr	HepaRG (24h)	8864	1986
Toxcast	MAO B	3711	831
PubChem	Carbonic Anhydrase Type I	**706**	**158**
PubChem	Carbonic Anhydrase Type II	2140	479
PubChem	Carbonic Anhydrase Type VI	14652	3282
Cell stress panel	HepaRG_3D (cell mem perm 168h)	9601	2197
HTTr	HepaRG_3D_24h	9538	2137

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Conclusions

Available tools can be integrated to make a safety decision

- NGRA is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions
- As applied here it is protective not predictive
- Need to ensure quality/robustness of the non-standard (non-TG) work and to characterise uncertainty to allow informed decision-making
  - Rethinking MoS/MoE
- Shortcomings will be addressed by current and future research
- More research, creativity and examples needed to land this successfully across the community

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